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TITLE: Targeting SRC Family Kinases and HSP90 in Lung Cancer

PRINCIPAL INVESTIGATOR: Erica Golemis, Ph.D.

CONTRACTING ORGANIZATION:

Institute for Cancer Research
Philadelphia, PA 19111

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Fort Detrick, Maryland 21702-5012

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5d. PROJECT NUMBER

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14. ABSTRACT

Lung cancer has the highest mortality rate of all cancers in the United States and has adversely affected the lives of many Americans. Typically, only 16% of patients survive 5 years beyond initial diagnosis. The goal of this proposal is to try to improve application of a drug, dasatinib, which has some promise for lung cancer treatment. Dasatinib is a targeted drug, with action that involves blocking the function of a group of proteins (defined as the Src group) whose action is important in lung cancer metastasis. In our study, we have been testing whether the action of dasatinib in lung cancer is enhanced by combining it with a second agent, ganetespib, that targets Src and other pro-cancerous proteins by an alternative mechanism. We have also been evaluating whether cellular status of a protein, NEDD9, that we have shown to bind directly to Src, influences the activity of dasatinib. Using in vivo analysis, experiments in progress are indicating that mice lacking NEDD9 are greatly sensitized to dasatinib but not ganetespib, and that mice lacking NEDD9 develop more aggressive lung cancers than those with intact NEDD9; while the dasatinib/ganetespib combination does not improve the efficacy of dasatinib.

15. SUBJECT TERMS
Nedd9, Src, HSP90, ganetespib, dasatinib, KRAS, NSCLC

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INTRODUCTION: Elevated activity of SRC family kinases (SFKs) and heat shock protein (HSP) 90 are both associated with cancer progression, invasion, tumor angiogenesis and drug-resistance, and both are targets of inhibitors currently in clinical development for the treatment of several cancers, including lung cancer. The scaffolding protein NEDD9 binds SFKs and controls their activity, and has very recently been defined as a factor regulating drug response and prognosis in lung cancer. The first evaluation of the efficacy of dual inhibition of SFKs and HSP90 lung cancer in the context of NEDD9 expression will be performed. The objectives of the proposal are to 1) Explore the therapeutic potential of combining dasatanib with ganetespib in lung cancer; 2) establish whether NEDD9 expression regulates response to dasatinib and ganetespib combination; and 3) define relevant related biomarkers for use in clinical assessment of response to the combination.

KEYWORDS: *Nedd9, Src, HSP90, ganetespib, dasatinib, KRAS, NSCLC.*

ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1 - Seven different lung cancer cell lines will be used to test the therapeutic potential of combining dasatinib with ganetespib in the context of different NEDD9 expression levels.

Major Task 2 – Utilize an *in vivo* lung cancer model to investigate the efficacy of combining dasatinib and ganetespib in the presence or absence of NEDD9.

For each goal, Reverse phase protein array (RPPA) will be performed to identify relevant signaling biomarkers.

What was accomplished under these goals?

For this reporting period, we have been working on the functional testing of NEDD9 genotype with dasatinib and/or ganetespib treatment for effects on cell viability in vitro (Major Task 1). Representative data illustrating one of the cell line models (H1299) are shown in Figures 1-5. For this, lung cancer cell lines were transfected with siNEDD9 or siControl. Experiments were performed in triplicate wells in a 96 well plate. Western analysis was routinely performed on pooled test samples to confirm effective knockdown of NEDD9; Figure 1 demonstrates that knockdown was typically by ~75-80%. These cells were then treated with vehicle, ganetespib, dasatinib or dasatinib plus ganetespib.

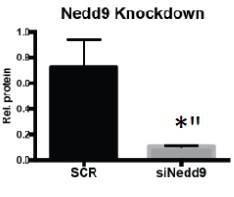

Figure 1. NEDD9-targeting siRNA is effective in depleting protein. *, p <0.05

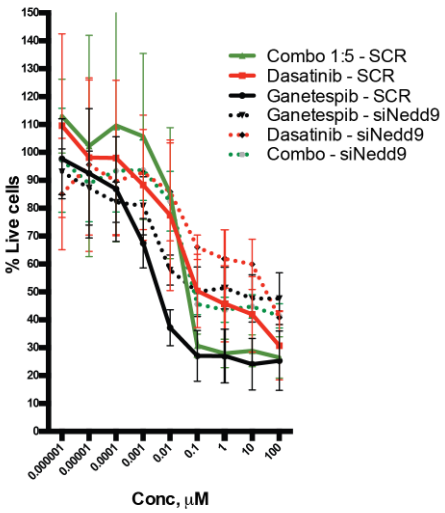

Figure 2. Reduction in cell viability relative to vehicle-treated control following indicated drug treatments, for SCR- or NEDD9-depletion.

In a typical experimental design, siRNA depletion was performed on day 0, and at 24 hours, drug was applied at 8 different concentrations, ranging from 1 pM through 100 μM. After an additional 72 hours, CellTiterBlue was used to measure cell viability. Figure 2 shows representative curves, averaged across multiple experiments. All data in the presented graph are normalized to cells treated with vehicle, normalized to either SCR or NEDD9 depletion. Based on the data in this cell line, we can make the following conclusions: 1) In control cells, treatment with ganetespib was effective in vitro, with an IC50 of 10 nM, and reducing the viability by 70% at 30-50 nM. 2) Dasatinib was less effective in these cells, with an IC50 of 100 nM, and total reduction of cell growth no more than 60% except at high concentrations where off-target activity

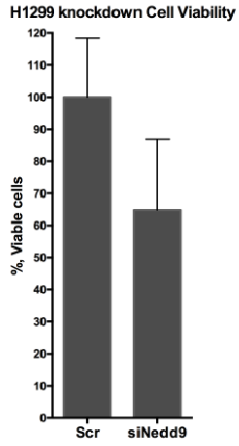

Figure 3. NEDD9-targeting siRNA reduces cell viability.

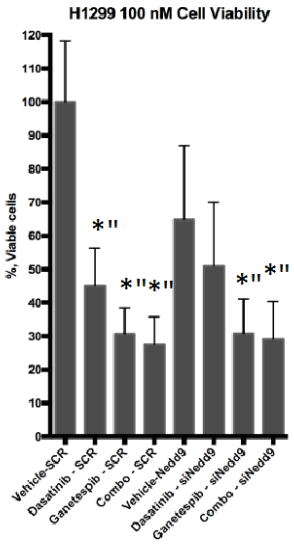

Figure 4. Interaction of NEDD9 depletion and response to drug treatment for viability. *, p <0.05 relative to veh/SCR

was expected. 3) The combination of ganetespib and dasatinib was not more effective than ganetespib; rather, IC50 values were intermediate between dasatinib and ganetespib. 4) NEDD9 depletion in the cells independently reduced viability by 35% (Figure 3). 5) In NEDD9-depleted cells, drugs were less effective. The maximum induction of loss of viability with any drug was 55% relative to starting values, complicating efforts to generate an IC50 value. 6) Although overall efficacy was less, the same pattern was observed, with ganetespib significantly more effective than dasatinib except at very high concentrations.

6) Figure 4 regraphs data from Fig 2 at 100 nM, now un-normalized to independent SCR- or NEDD9-depleted, vehicle-treated controls, but all normalized to SCR/vehicle controls. This data indicates that in cells with depleted NEDD9, although the cell viability endpoint was comparable to SCR-depleted cells, the efficacy of the drugs tested is proportionally reduced, particularly for treatment with dasatinib. Similar experiments are being conducted for the other cell models, with repetitions in progress to obtain statistically robust data with tighter error bars.

We are performing signaling analysis. The longer-term strategy has been to send specimens for RPPA analysis; we are still accruing these, so it is not possible to report on all the data at this time. The complete set of specimens should be processed by spring 2016. As an interim assessment of drug activity we have been performing basic signaling characterization of the direct target of dasatinib (SRC kinase) but also is dependent on interactions with HSP90 chaperone for activation of AKT, which is known to reflect HSP90 activity, and hence provides an indirect indication of both ganetespib activity and cell viability. As shown in representative Figure 5, depletion of NEDD9 per se causes a very significant decrease in the basal activity of SRC. This is associated with a different pattern of response between SCR and NEDD9-depleted cells; whereas dasatinib and ganetespib have some activity in SCR-depleted cells, both are inactive in NEDDD9-depleted cells. In both conditions, degree of SRC inhibition is greater with the drug combination than with drugs used alone. In both conditions, treatment with any drug resulted in a modest elevation of total SRC protein, most likely in compensatory feedback, although response is heterogeneous and does not achieve statistical significance (not shown). For AKT, a distinct and somewhat surprising pattern was observed, with depletion of NEDD9 or treatment with ganetespib (particularly in the context of NEDD9 depletion) significantly inducing AKT phosphorylation. This may reflect the fact that NEDD9-depleted cells are undergoing stresses associated with induction of apoptotic signaling, compatible with reports in the literature. We are performing further repetitions of these studies.

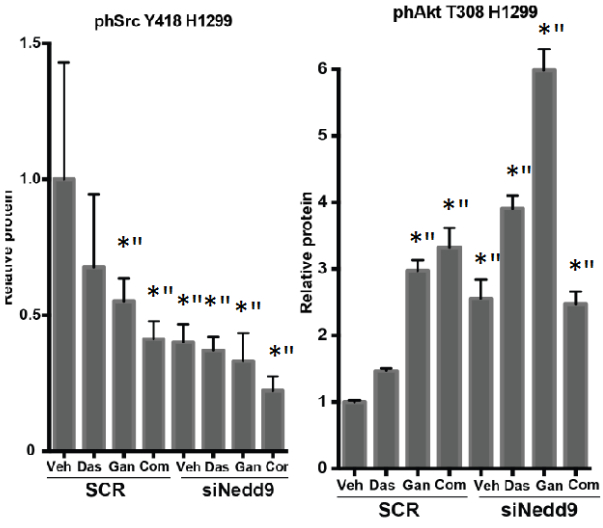

Figure 5. Interaction of NEDD9 depletion and response to drug treatment for activity of SRC (left) and AKT (right) as indicated by activity-associated phosphorylation. *, p <0.05 relative to veh/SCR

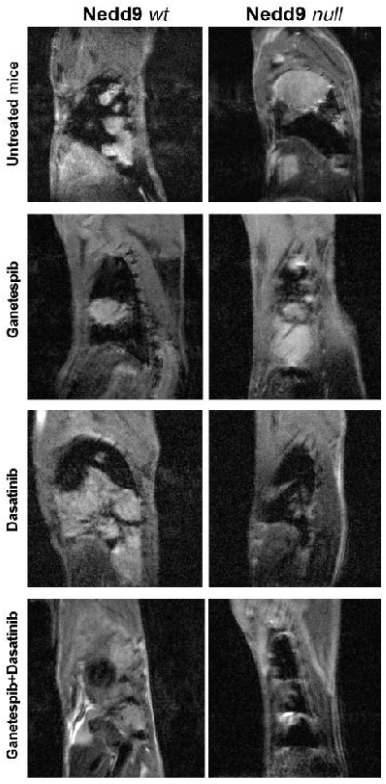

Figure 6. Representative MRI images from Nedd9wt or Nedd9 null Kras mutant mice, treated with indicated drugs.

We have completed in vivo analysis of the mouse cohorts needed to assess the effect of NEDD9 genotype with dasatinib and/or ganetespib treatment

for effects on tumor growth (Major Task 2). This involved inducing tumor formation in 129S/Sv-*Kras*^{tm3Tyj}/J;Nedd9$^{+/+}$ (subsequently called Nedd9 wt) mice and 129S/Sv-*Kras*^{tm3Tyj}/J;Nedd9$^{-/-}$ (subsequently called Nedd9$^{-/-}$ or Nedd9 null) mice via inhalation of Adeno-Cre, followed by imaging at regular intervals to detect tumor initiation and growth, followed by euthanasia and processing of tissues for mechanistic analysis. Representative images of MRI are shown in Figure 6. Quantification is shown in Figures 7 and 8.

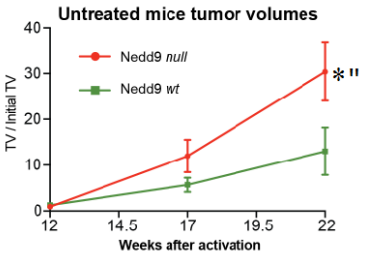

Figure 7. Tumor volume (TV) differences over time associated with Nedd9 genotype. *, p <0.05

We have reached the following conclusions: 1) There is a robust effect of NEDD9 genotype on tumor growth in untreated mice. Null status for Nedd9 resulted in an increase in tumor burden by more than 200% at 22 weeks after tumor induction, relative to Nedd9 wild type mice (Figure 7; data reflects full proposed cohorts of mice for all experiments). This was extremely unexpected: our previous work with Nedd9 knockout in a mammary tumor model showed the opposite result, with significantly reduced tumor growth. 2) We have detected a robust effect of NEDD9 genotype on response to dasatinib. Treatment of Nedd9$^{-/-}$ mice with dasatinib resulted in near elimination of tumors, with essentially no growth observed after 22 weeks. A similar effect was observed with the dasatinib/ganetespib combination treatment (Figure 8A). In contrast, treatment of the Nedd9 wt mice with dasatinib reduced the rate of tumor growth, but this was non-statistically significant (Figure 8B). 3) Ganetespib treatment reduced the rate of growth of tumors in this model. Although the average degree of reduction was marked (70-80% less in ganetespib-treated versus vehicle-treated at 22 weeks), because of heterogeneity of response, this result did not quite attain statistical significance, although we suspect it would be with larger cohorts. We did not detect an effect of Nedd9 genotype on response to ganetespib, nor did we detect improved tumor control with the ganetespib/dasatinib combination, regardless of Nedd9 genotype.

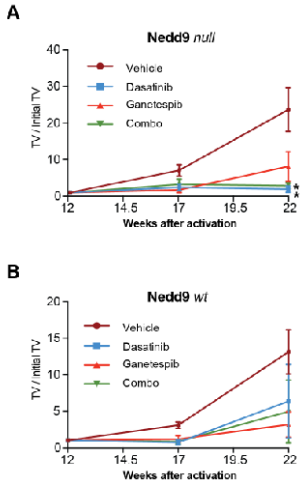

Figure 8. Differences in tumor volume (TV) based on the interaction of drug treatment and Nedd9 genotype. *, p <0.05

We are currently performing histopathological assessment of tumor specimens from tissues that have been collected and paraffin embedded. Representative hematoxylin and eosin stained specimens are shown in Figure 9. A notable feature of this analysis is that while Nedd9 wt lungs have multiple small tumors detectable, these are uniformly confined within the area of the lung. The Nedd9 null mice, by contrast, have very large tumors in the lung, and evidence in some cases of metastasis to neighboring tissues, such as the heart. Slides have been cut to support the use of all markers proposed. We have stored specimens to send to for RPPA analysis (estimated January 2016, to avoid potential problems shipping material over the holidays). Based on this work, together with our signaling studies, and based on the recent withdrawal of ganetespib from a phase 3 clinical trial due to lack of efficacy (late October 2015), we view the most important result emerging from this study as revealing the activity of NEDD9 in strongly effecting tumor growth and response to SRC inhibition, and are prioritizing future analysis of the underlying signaling mechanism.

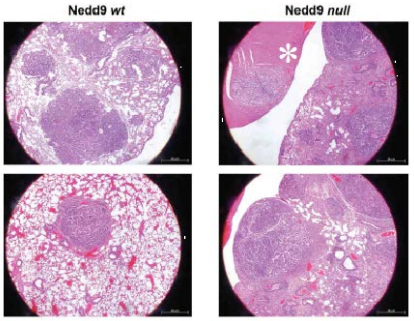

Figure 9. Representative H&E stained lung tissue in 2 Nedd9 wt versus 2 Nedd9 null animals. * in top right panel indicates metastasis to adjacent heart tissue.

What opportunities for training and professional development has the project provided?

This project provided extensive training and professional development to involved staff. Anna Gaponova (in a Ph.D. training program) received extensive training in analysis of MRI imaging and performance of xenograft experiments involving assessment of drugs. She also provided training to multiple undergraduates working in the laboratory over the past summer, who shadowed her in the performance of experiment. She has communicated these skills to other laboratory staff, including 1 postdoctoral fellow and 1 MD/PhD student. She

has worked with the PI of the project to learn how to analyze and present the resulting data in research papers and seminars.

How were the results disseminated to communities of interest?

Nothing to report: results will be disseminated in the forms of publications and presentation at meetings in 2016.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we will finish the proposed research plan as described. This includes repetition of in vitro analyses, performance of RPPA analysis for in vitro and in vivo data, and histopathological assessment of tumors.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The data strongly suggest that NEDD9 genotype conditions response to dasatinib in lung cancer. When the result is reported (2016) this is likely to validate NEDD9 expression as a useful biomarker for use in treatment with agents targeting the SRC pathway.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

The reason the project was delayed in commencing was slow breeding by the mice, resulting in a longer time than anticipated to generate the proposed cohorts for in vivo testing Major Task 2. We anticipate no further delays, as all mice required are now available or have already been analyzed.

Changes that had a significant impact on expenditures

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None.

Significant changes in use or care of human subjects

Not applicable, no human subjects

Significant changes in use or care of vertebrate animals.

None.

Significant changes in use of biohazards and/or select agents

None.

PRODUCTS: Nothing to Report.

Publications, conference papers, and presentations

Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	***Erica Golemis, Ph.D.***
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	egolemis
Nearest person month worked:	*2*
Contribution to Project:	Overall administration and guidance of the research; Training and management of personnel involved
Funding Support:	*Partial salary support is covered by institutional sources.*
Name:	***Anna Gaponova***

Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7*
Contribution to Project:	*In vitro* and *in vivo* experiments proposed; Mouse work
Funding Support:	Not applicable

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Please see Other Support attached. Changes from the grant submission are marked with a line in the right hand margin.

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS: None.

Collaborative Awards: Not applicable

Quad Charts: Not applicable

APPENDICES: None

Other Support - Golemis, Erica A.
Remaining salary support from institutional sources.

ACTIVE

P30 CA006927 (PI: Fisher) 7/21/2011 - 6/30/2016 20.0%
NIH Partial Salary 2.40 calendar
Comprehensive Cancer Center Program at Fox Chase
The major goal of this Cancer Center Support Grant is to provide partial salary support for professional personnel, including senior and program leadership, administration, planning and evaluation, and developmental funds, as well as support for 4 established peer-reviewed Research Programs, 12 Shared Research Resources and 1 Support Element.
Procuring Contracting/Grants Officer: Emily Tran, OGA 9609 Medical Center Dr., West Tower, 2nd Flr., Rockville, MD 20850, 240-276-6324

R56 DK108195 (PI: Golemis) 9/17/2015 - 8/31/2016 5.0%
NIH $65,000 (No Salary) 0.60 calendar
Interaction of Protein-targeted Therapeutics and Ciliary Dynamics
The major goals of this project are to: 1) To sustain mouse colonies; 2) To perform experiments assessing metformin activity in limiting cystic growth in a late induction model for ADPKD; 3) To demonstrate that renal cell cultures are epithelial in nature, and investigate response to metformin and lisinopril; and 4) To analyze the results from experiments described in Aims 2 and 3.
Procuring Contracting/Grants Officer: Krystle Nicholson, 6707 Democracy Blvd., BG 2DEM, RM 735C, Bethesda, MD 20817, 301-594-8860

R21 CA181287 (PI: Golemis) 7/7/2014 - 6/30/2016 10.0%
NIH $130,760 1.20 calendar
A Role for AMH Autocrine Signaling in NSCLC
The major goals of this project are: 1) To dissect AMH/AMHRII signaling in NSCLC cells, emphasizing the context of HSP90 inhibition; and 2) To evaluate AMH and AMHRII as therapeutic targets and biomarkers.
Procuring Contracting/Grants Officer: Emily Tran, OGA 9609 Medical Center Dr., West Tower, 2nd Flr., Rockville, MD 20850, 240-276-6324

186g14a (PI: Golemis) 3/1/2014 - 2/29/2016 10.0%
PKD $80,000 1.20 calendar
Advancing HSP90 Inhibitors towards Clinical Trials for ADPKD
The major goals of this project are: 1) To evaluate efficacy of STA-2842 in limiting the growth of PKD2-dependent ADPKD in a mouse model; 2) To evaluate the tolerability and efficacy of HSP90 administration over a long time period; and 3) To assess whether a combination of 2DG and STA-2842 shows enhanced activity in limiting cyst formation and decline in kidney function.
Procuring Contracting/Grants Officer: Leslie White, 8330 Ward parkway, Suite 510, Kansas City, MO 64114, 800-753-2873x148

W81XWH-14-1-0135 (PI: Golemis) 9/30/2014 - 9/29/2016 20.0%
Army $23,367 (Partial Salary) 2.40 calendar
Targeting SRC Family Kinases and HSP90 in Lung Cancer
This grant is in a a no-cost extension.
The major goals of this project are: 1) To explore the therapeutic potential of combining dasatanib with ganetespib in lung cancer; 2) To establish whether NEDD9 expression regulates response to dasatinib and ganetespib combination; and 3) To define relevant related biomarkers for use in clinical assessment of response to the combination.
Procuring Contracting/Grants Officer: Cheryl Lowery, USAMRAA, 820 Chandler St., Fort Detrick, MD, 21702-5014, 301-619-7150

U54 CA149147 (PI: Clarke, Georgetown Univ.) 4/29/2010 - 2/29/2016 4.0%
NIH $599 0.48 calendar
Integration of ER-related Signaling in Breast Cancer Partial Salary
This grant is in a one year extension.
This project is a subcontract to Georgetown University.
Procuring Contracting/Grants Officer: Dan McDermott, Lombardi Cancer Center, 3520 Prospect St., NW, Suite 312, Washington, DC 202-687-2936

COMPLETED
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R01 CA050633
P50 CA083638
K99 CA158065
R21 CA164205
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K22 CA160725